

## Patent Claims

1. A composition or reaction mixture for performing multi-color real time PCR comprising at least 3, preferably 4-5 most preferably exactly 4 pairs of FRET hybridization probes, each pair of hybridization probes consisting of a FRET donor probe carrying a FRET donor moiety and a FRET acceptor probe carrying a FRET acceptor moiety having an emission maximum between 550 and 710 nm.  
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2. A composition or reaction mixture according to claim 1, wherein at least 3, preferably at least 4 and most preferably exactly 4 FRET donor moieties are identical.  
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3. A composition or reaction mixture according to claim 1, wherein all FRET donor moieties are identical.
4. A composition or reaction mixture according to claim 1, wherein at least 3, preferably at least 4 and most preferably exactly 4 FRET donor moieties are Fluorescein.  
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5. A composition or reaction mixture according to claim 1, wherein all FRET donor moieties are Fluorescein.
6. A composition or reaction mixture according to claims 1-5, wherein at least one additional FRET donor moiety is selected from a group consisting of Atto425 and WI343.  
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7. A composition or reaction mixture according to claims 1-6, wherein one FRET acceptor moiety is selected from a group consisting of LC-Red 705, Cy5.5, and JA286.
8. A composition or reaction mixture according to claims 1-7, wherein at least one, two or three FRET acceptor moieties are selected from a group consisting of Cy5, LC-Red 640, and LC-Red 610  
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9. A composition or reaction mixture according to claims 1-8,

wherein one FRET acceptor moiety is selected from a group consisting of Rh6G and TAMRA.

10. A system for performing multi-color real time PCR, comprising

- 5      ◦ a real time PCR instrument, and
- a composition or reaction mixture according to claims 1-9.

11. A system according to claim 10, characterized in that said real time PCR instrument comprises

- 10      • at least 1 light source, preferably an LED
- at least 4 and preferably 5-6 fluorescent detector entities, each of said entities having central detection wavelengths which are distinct from each other by at least 25 and preferably at least 30 nm

characterized in that said detector entities are capable of

- 15      • simultaneously detecting maximum fluorescene emission of at least 3, preferably 4 and most preferably 5 differently labeled FRET Hybridization Probe pairs,
- simultaneously detecting maximum fluorescence emission of at least 2 differently labeled TaqMan hybridization probes, and
- detecting maximum fluorescence emission of SybrGreenI

- 20      • means for heating and cooling
- multiple reaction vessels for containing a reaction mixture.

12. A system according to claim 11, characterized in that said real time PCR instrument comprises exactly one light source.

13. A method for amplifying and detecting multiple target DNA sequences comprising

- 25      a) providing a composition or reaction mixture according to claims 1-9,

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- b) subjecting said reaction mixture to a thermocycling protocol such that amplification of said multiple target sequences can take place,
- c) monitoring hybridization of each of said pairs of FRET hybridization probes at least once after a plurality of amplification cycles.

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14. A method according to claim 13, wherein hybridization is monitored at least once in a temperature dependent manner.

15. A real time PCR instrument comprising

- at least 1 light source, preferably an LED
- 5-6 fluorescent detector entities, each of said entities having central detection wavelengths which are distinct from each other by at least 25 and preferably at least 30 nm,

characterized in that said detector entities are capable of

- 15 • simultaneously detecting maximum fluorescene emission of at least 3, preferably 4 and most preferably 5 differently labeled FRET Hybridization Probe pairs,
- simultaneously detecting maximum fluorescence emission of at least 2 differently labeled TaqMan hybridization probes, and
- detecting maximum fluorescence emission of SybrGreenI

  

- means for heating and cooling
- multiple reaction vessels for containing a reaction mixture.

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16. A real time PCR instrument according to claim 15 comprising exactly one light source.

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17. An instrument according to claim 15-16, characterized in that said central detection wavelengths are selected from a group of range of wavelengths, said group conisiting of 520-540 nm, 545-565 nm, 570-590 nm, 600-620 nm, 630-650 nm, 660-680 nm, and 700-720 nm.